UNITED STATES DEPARTMENT OF AGRICULTURE

FOREST SERVICE

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LABORATORY CHARACTERIZATION OF *BACILLUS THURINGIENSIS* SPRAY MIXTURES TO BE APPLIED TO CONTROL WESTERN SPRUCE BUDWORM

BY

JOHN NEISESS

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STUDY PLAN SUMMARY FORM
Copy 1 - Project files
Copy 2 - Route:
AD or Program Manager ________
Information Office ________
P&A AD ________

A. The Study:

Title Laboratory characterization of Bacillus thuringiensis spray mixtures to be applied to control western spruce budworm

Study Leader John Neisess Identifier PNW 2203-78-1

Project Leader C. G. Thompson Date Approved ________

Cooperators None

Study Location FSL Corvallis, Oregon Termination date 1980

Objective(s): (1) To evaluate and characterize how rain and sunlight affect the residual activity of Bt spray mixtures; (2) To evaluate adjuvants affect on activity of Bt spray deposits.

Justification Weathering factors may affect the efficacy of Bt sprays against forest insects. Spray formulation - insect interactions need to be studied for each forest pest to determine the best spray mixture.

B. Audiences:

X Forest or range resource managers

____

(Be specific)

___ General public
___ Key decision makers
X Other Scientists

C. Anticipated Impact:

___ Controversial
X Noncontroversial
X National
___ Local

D. Technology Transfer:

1. Scientific or technical publication
J. Econ. Entomology - Neisess
(Indicate name of journal and anticipated author)

2. Suggestions for other techniques — Who should prepare (scientists and/or information office, other)

___ Trade journal ________
___ Newspaper story ________
___ Magazine article ________
X How-to guide or handbook on some ~~of the techniques~~ ________
___ Slide talk ________
___ PNW brochure ________
___ Summary publication ________
___ Self-guided tour ________
___ Other ________
___ Workshop or seminar

Check one

___ Information stands alone
X Needs integration with other scientific information for technology transfer purposes.

E. Reviewers Comments

Title: Laboratory Characterization of <u>Bacillus thuringiensis</u> Spray Mixtures to be Applied to Control Western Spruce Budworm

Principal Investigator: John Neisess

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SBW RD&A Program Activity No.: 3.2.1

Approved by:

C. G. Thompson Date 2-21-78
C. G. Thompson, Project Leader, RWU 2203

R. M. Romancier Date 2/23/78
Robert M. Romancier Assistant Director

M. W. McFadden Date 10/27/78
M. W. McFadden, Program Manager

Reviewed by:

Milton J. Stelzer Date 2-20-78
Milton J. Stelzer

Russel G. Mitchell Date 5/25/78
Russel G. Mitchell

John Hazard Date 10/24/78
John Hazard, PNW Biometrician

STUDY SUMMARY FORM

Study Identifier PNW-2203-78-1 SBW RD&A activity 3.2.1

Date Approved Feb. 23, 1978

Study Title Laboratory characterization of Bacillus thuringiensis spray mixtures to be applied to control western spruce budworm

Study Leader John Neisess

Project Leader C. G. Thompson

Cooperators (if any) None

Study Location FSL, Corvallis, Oregon

Expected Duration 2 years

Objective(s) (1) To evaluate and characterize how rain and sunlight affect the residual activity of Bt spray mixtures to be applied against western spruce budworm. (2) To evaluate how adjuvants affect the activity of Bt spray mixtures. Both initial and residual activity will be evaluated in terms of weatherability (rainfastness) and inactivation

~~Additional Information~~ by sunlight. (3) To evaluate how the Bt formulation (wettable powders vs. water-dispersable liquids) affects residual activity.

Termination Date 1980

PNW 4000-1
July 1972

LABORATORY CHARACTERIZATION OF BACILLUS THURINGIENSIS SPRAY MIXTURES TO BE APPLIED TO CONTROL WESTERN SPRUCE BUDWORM

OBJECTIVES

Overall Objective

To develop spray mixtures that can be used to apply *Bacillus thuringiensis* (Bt) for control of western spruce budworm, *Choristoneura occidentalis* Freeman (WSB).

Specific Objectives

(1) To evaluate and characterize how rain and sunlight affect the residual activity of Bt spray mixtures to be applied against western spruce budworm.

(2) To evaluate how adjuvants affect the activity of Bt spray mixtures. Both initial and residual activity will be evaluated in terms of weatherability (rainfastness) and inactivation by sunlight.

(3) To evaluate how the Bt formulation (wettable powders vs. water-dispersable liquids) affects residual activity.

These objectives obviously cover a multi-year research effort. A certain portion of the laboratory work will be involved with spray mixtures that are destined for immediate field testing. These mixtures, however, may not be the optimum tank mixes. In FY 78, this would include evaluations of two formulations destined for field use: wettable powder and water-dispersable liquid formulations mixed in 25% molasses. Evaluations of sticking, feeding stimulation, evaporation, and inactivation by sunlight will be initiated in FY 78, but many studies will not be completed for one or two years.

JUSTIFICATION

Chemical and microbial insecticides which have been applied in forestry primarily use formulations and application techniques developed for agriculture. However, the problems encountered in forest applications are much more complex than those found in agriculture. Spraying heights are 50 or more feet above the crop; therefore, the spray particles are airborne for longer durations, which increases the effects of evaporation, oxidation-reduction, and inactivation by sunlight. Forestry applications are generally made only once in a season, whereas multiple applications is the rule in agriculture. Therefore, the problems of coverage and residual activity become more important for forest applications.

Improvements can be made in the spray mixtures with the addition of spray adjuvants which will reduce evaporation and inactivation by sunlight and improve sticking. However, each spray mixture or addition of adjuvant to a spray mixture should be evaluated against western spruce budworm because the interaction between Bt and adjuvant may be different for specific insects. For instance, we know that increased coverage can reduce residual activity since small droplets (generally indicating increased coverage) are inactivated faster than large droplets (Thompson et al. 1978), but we do not know the relative importance of coverage or residual activity for WSB.

Currently we do not know which Bt spray mixture should be applied against WSB. Region 1 applied Dipel WP mixed in 0.125% BioFilm at the rate of 2 gal/acre and reported only moderate population control but good foliage protection (McGregor, M. P., personal communication).

However, our data showed that 25% molasses and 25% Sutro + Shade[R] mixtures were both superior to BioFilm, but neither mixture provided sufficient WSB population control (Thompson et al. 1978). The importance of UV inactivation, sticking, and sugar additives (molasses) needs to be determined in the laboratory before field experiments can be successfully designed to test for efficacy.

CURRENT KNOWLEDGE

Various spray adjuvants have been added to microbial spray mixtures to make them more suitable for field use and to increase the field effectiveness of the microbial insecticide (Angus and Luthy 1971). In many instances, these adjuvants have been added to a microbial spray mixture to be used for a given insect because of desirable effects resulting from a specific microbial-spray mixture combination for a different insect, or because a specific adjuvant worked well with a chemical insecticide. Studies need to be conducted to characterize the interactions of each insect-microbial-spray mixture combination.

Most publications dealing with formulation of microbial insecticdes deal only with the active ingredient and do not consider the host insect or the interaction of insect and spray mixture. For example, Morris (1975) has reported that certain emulsifiers, such as Atlox 1971 and Triton-X-100, are toxic to Bt by inhibiting Bt germination and growth. Since the relative importance of Bacillus thuringiensis (Bt) spores and crystals may change from one insect to the next, the effects that spray adjuvants may have on Bt efficacy against WSB may be different than effects on efficacy against Douglas-fir tussock moth. Molasses, a common additive to Bt spray preparations, illustrates how the effect

of adjuvant can change from one insect to the next. Yendol et al. (1975) found that molasses stimulated feeding of gypsy moth on Bt treated foliage. Although Douglas-fir tussock moth showed no preference to feeding on untreated or Bt + molasses-treated foliage (Stelzer, personal communication), the addition of molasses to the spray mixture did increase the effectiveness of the Bt suspension (Neisess 1977). Obviously, the molasses-insect interaction is different for tussock moth than for gypsy moth. The sunscreen, Shade^{R}, may also exhibit a differential spray additive-insect interaction. Results of a field experiment evaluating Bt spray formulations showed that Shade prolonged the field activity when bioassayed against tussock moth. Yet, no differences were noted in western spruce budworm mortalities with the same addition of Shade to the spray mixture (Thompson et al. 1978). However, since laboratory experiments by Thompson and Yusha (personal communication) show that Bt spores play a very important role in insectial activity against western spruce budworm, protecting the spores from inactivation from sunlight seems to be an important factor.

EXPERIMENTAL DESIGN

Factorial experiments will be used almost exclusively in these laboratory studies. This type of design allows for proper interpretation of the interaction between additives or between additives and commercial Bt products which may be different. Changes in physical properties (pH, emulsion stability, evaporation, viscosity, density, surface tension) will first be studied as a function of single additives and then as a function of multiple additives. Multiple effects will be limited to spray mixtures which are judged to be ready for field testing. Every measurement or treatment will be replicated 5 times.

What kind of analysis is planned?

MATERIALS AND METHODS

Materials, in the form of specific adjuvants, should not be limited to those which can be listed in this study plan. All adjuvants which were tested with Douglas-fir tussock moth should eventually be tested to some degree with WSB. Bt products will be limited to Dipel[R] and Thuricide[R] products. Biotrol[R] products will not be included in this experiment because of uncertainties in the future of this product. The methods outlined in this study have been used with Douglas-fir tussock moth and may require modifications for use with WSB. New methods and specific test compounds will be included as addendums to this plan. The physical parameters of most additives have already been characterized as part of the DFTM program. Only pest or host related studies need to be completed.

pH

A model 10 Corning pH meter equipped with a triple purpose Ag/AgCl glass and Calomel fiber type reference electrodes will be used to measure the formulations. pH will be measured for single additivies + water and

combinations of additives + water. Measurements will be made at additive concentrations normally used in spray mixtures (for example, 1 ml/100 ml for stickers). If the pH of the solution is greater than 9.5 and can't be readily buffered to a lower pH, this pH effect will have to be tested further.

Physical Stability

Our method for determining the stability of the emulsions is a modified Collaborative Pesticide Analytical Committee of Europe (CPAC) method (Ashworth 1966). The spray mixtures will be placed in 100 ml stoppered, graduated cylinders, agitated by inverting the cylinder 10 times and held for 24 h in a waterbath at $30^{\circ} \pm 1^{\circ}C$. Inspections will be made after 0.5, 2, and 24 h, and the amount of separation or layering will be measured. Bioassays will be conducted on aliquots sampled from each layer to determine insecticidal activity of each layer. (Time will not permit bioassay of all formulations, but a representative sample will be tested to indicate how physical stability is related to biological activity.) Media surface contamination methods will be used for bioassay (Neisess 1977b).

Viscosity

A Brookfield viscometer will be used for all viscosity measurements. Viscosities will be determined at various rates of shear, concentration of additives (constant shear), time, and temperature.

Specific Gravity

Gay Lussac specific gravity bottles will be used to determine densities for liquids and a Le Chatelier bottle will be used for solids. Specific gravities will be converted to lb/gal for spray mixtures which will be field tested to assist in payload calculations.

Surface Tension

Surface tensions will be measured with a Rosano (TM) surface tensiometer which measures force (f) in milligrams directly. Surface tension is calculated from:

$$\text{Surface tension (dynes/cm)} = \delta = \frac{F \times 0.980}{W}$$

Where W = perimeter of blade = 5 cm (for our blade)

Evaporation

Relative evaporation will be determined by a method similar to that described by Hartley (1969). Five 10 ml replicates of each material (including water) will be placed in petri dishes--(264.5 cm^2). Dishes will be weighed at 10 min, 30 min, 1 h, and 24 h intervals. The temperature will be kept at 25^{o}C and 40% relative humidity (RH). Volume loss calculated from weight loss will give relative evaporation.

Sticking

The 18 sticking agents which will be evaluated in this experiment are listed in Table 1. New stickers will be added to the list as they are acquired. These stickers will be combined in 3 aqueous mixtures: water, 25% molasses, and 25% Sutro^{R}, and with 2 commercial Bt products: Dipel^{R} 36B and Thuricide^{R} 32. More mixtures of Bt product will be added if the need arises. Thuricide 32B mixtures were also compared to a mixture of Thuricide 16B mixed with 1:1 with water. Thuricide 16B is a preformulated product which is supposed to contain sticking agents. The Bt concentrations will be 5.28 X 10^5 international units (IU/ml) (equivalent to 2 X 10^9 IU/gal - 1/4 the usual field concentration). Sticker concentrations will be 1% by volume except for Polyhall^{R} 295 and Carboset^{R} 525, which are solids. These

two stickers will be mixed at the rate of 0.125 g/100 ml and 1.0 g/100 ml, respectively. The lower rate is used with Polyhall 295 because of thickening properties of this compound; higher rates are too viscous to pass through spray equipment.

The same mixing order and procedure will be used with all stickers and treatment combinations. Sticker will be added to the proper amount of water to make 100 ml of final mix. This mix will be agitated until the sticker has either dissolved or become completely suspended (emulsified). (Since Carboset 525 requires an alkaline solution for solubility, 0.5 ml of ammonium hydroxide will be added to the water. Molasses or Sutro will be added at the rate of 25 ml/100 ml if either of these carriers is specified.) After agitating the above mixture, the proper Bt product will be added and agitated again. At this point, any precipitation or formulation of water-insoluble films will be noted. This part of the experiment has already been completed in the DFTM program for the stickers listed in Table 1.

What pH?

drop size?

The effectiveness of the sticking agents will be evaluated as follows: 0.1 ml replicates of each treatment will be sprayed onto Douglas-fir cuttings, Pseudotsuga menziesii (Mirb.) Franco. Only new growth foliage will be used for the bioassay. The spray residues will be allowed to dry overnight (20 to 24 hr) in total darkness at room temperature (20-22°C). A pre-rain sample (25 cm total length) will be cut from each treated cutting and placed in a 7 oz specimen container with a paper lid. The remaining portion of the treated cutting will be exposed to simulated rain. After exposure to 0.5 in and 1.0 in of rain, samples will be cut and placed in petri dishes the same as the pre-rain samples. Twenty 4th instar western spruce budworm larvae from our laboratory colony will be

also need shorter drying times – 2-4-6 hrs

These may be standard tests but they do not represent likely field conditions

exposed to untreated foliage which has been exposed to rain the same as the treated foliage. Five replicates (20 larvae/container/replicate) will be tested with each treatment at each rain exposure. Mortality will be recorded after the larvae are held at 25.5 $\pm$ 1.0^{o}C and 40 $\pm$ 5% relative humidity (RH) for 96 h. The percentage of the original activity of the Bt mixture remaining (OAR) after exposure to rain is based upon the mortality of the pre-rain samples. Larval mortality and OAR will be used to measure weatherability of the treatments.

<u>Inactivation by sunlight</u>

Artificial sunlight will be provided by a bank of four General Electric lamps, two F-30-T8 BL and two G-30-T8, built into a special exposure chamber. The lamps are arranged alternately, axis to axis, 7.5 cm apart. Exposures will be conducted at 24^{o} $\pm$ 2^{o}C with 65% RH. The energy of short-wave UV (Range 215-260 nm) and long-wave UV (Range 290-400 nm) measures 1600 μw/cm^2 and 700 μw/cm^2, respectively. Natural summer sunlight, UV-energy measured between 2 and 3 p.m. at Corvallis, Oregon averages 4125 μw/cm^2 and 1500 μw/cm^2 for long- and short-wave UV respectively. A Model J-221 (long-wave) and Model J-225 (short-wave) Blak-Ray UV meter (Ultraviolet Products, Inc., San Gabriel, CA) is used to measure ultraviolet intensities.

Specifically identify mechanism for inactivation?

The effectiveness of the compounds as sunscreen agents will be studied by using a bioassay technique incorporating Douglas-fir foliage as in the sticking experiment. Five 0.1 ml aliquots of each test material will be sprayed onto Douglas-fir cuttings (9- to 12-cm long) using a S.T.4 Laboratory Spray Tower (Barkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, England). Pre-UV-exposure samples (ca 25 cm long) taken

what drop size?

from treated and untreated (untreated control) Douglas-fir cuttings, after the spray residues have air dried, will be placed individually in 50 X 90 mm tight lid petri dishes. The remainder of each treated cutting, as well as the untreated controls, are then exposed to the sunlight source. After exposure for 16 and 24 h (this time may change), samples (ca 3 cm long) are collected from each cutting and placed in each petri dish. Ten 4th instar WSB larvae are placed in each petri dish. Mortality is recorded after the larvae are held at 25 $\pm$ 2^{o}C and 40 $\pm$ 5% RH. Five replicates of 10 larvae/replicate will be tested with each spray mixture and exposure period. The percent original activity (OAR) of the Bt is based upon the mortality of larvae placed on the pre-exposure samples.

Feeding Repellency or Stimulation

Foliage samples will be randomly cut from potted seedlings (3-4 yrs), weighed, and treated with either single ingredients, complete formulations, or left as untreated control. Three 4th instar WSB larvae will be bio-assayed on weighed foliage for 4 days. After 4 days, the larvae will be placed on artificial media and reared for an additional 14 days to check for mortality. Feeding will be evaluated by wet weight consumed and oven-dried frass weight (frass will be collected only while insects are feeding on the foliage).

WORK SCHEDULE AND MANPOWER REQUIREMENTS

Evaluation of spray mixtures which are planned for field testing against WSB this field season will begin about mid-February. Initial studies dealing with sticking and UV inactivation will begin sometime in March. Companies that supply anti-evaporants will be contacted to get more samples. As new samples (materials) are added to this total

design, the entire scope of the laboratory studies may change. Such changes will necessarily require either new study plans or addendums to this plan.

Manpower:	Obligation:
One GS-12 Scientist	1/2
One GS-4 Technician	full time

I will also be getting assistance and cooperation from Milton Stelzer (GS-13 Entomologist), and Lu Clark (GS-5 Laboratory Technician).

HAZARD ANALYSIS

All laboratory personnel will be briefed in overall laboratory safety. Lab coats should be worn at all times to protect personal clothing. Special precautions should be used when operating the spray tower and simulated light chamber. The hood connected to the spray tower should be turned on any time the tower is being operated to exhaust aerosol spray particles. When the light chamber is being used, the front shield should always be in place. If the shied needs to be removed while the UV lights are on, UV safety goggles will be worn by the operator.

Budget Estimates (Oct. 1, 1978 - Apr. 30, 1979)

	PNW	WSB Program
a. Salaries and wages		
John Neisess (75%)	$11,058	--
	--	4,453
Total	$11,058	$5,453
b. Supplies		
Petri dishes	--	1,000
Plastic cups	--	500
Misc. (bench, jelly cups, etc)	--	1,000
WSB media	--	500
Total	0	$3,000
c. Travel		
General	--	1,000
d. Overhead	3,686	3,151
TOTAL COSTS	$14,744	$12,604

BUDGET

Budget Estimates (Apr. 1 - Sept. 30, 1978)

	PNW	WSB Program
a. Salaries and wages		
John Neisess (75%)	$8,753	---
GS-4	--	$4,450
Total	$8,753	$4,450
b. Equipment		
Greenhouse lights	--	300
Petri dishes	--	1,000
Plastic pipets	--	100
Plastic cups (for budworm rearing)	--	500
General (glassware, ParaFilm, etc)	--	500
Total	0	2,400
c. Supplies		
WSB media	--	500
Douglas-fir seedlings	--	50
Total	0	$550
d. Travel		
General	--	$1,000
Chicago meeting (Neisess & Orchard)	--	772
Total	0	$1,772
e. Overhead		
25% for Station	2,918	3,058
TOTAL COSTS	$11,671	$12,230

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Yendol, W. G., R. A. Hamlen, and S. B. Rosario. 1975. Feeding behavior of gypsy moth larvae on *Bacillus thuringiensis*-treated foliage. J. Econ. Entomol. 68: 25-27.

Table 1. List of sticking agents and their sources.

STICKERS	COMPANIES
Adsee 775	Witco Chemical Corp.
Bio-Film	Colloidal Products Inc.
Carboset® 514H	B.F. Goodrich Chemical Co.
Carboset® 525	B.F. Goodrich Chemical Co.
Chevron Spray Sticker	Chevron Chemical Co.
Exhalt™ 800	Kay-Fries Chemical Inc.
Geon 552	B.F. Goodrich Chemical Co.
High Tack Fish Glue	Norland Products Inc.
Hycar 1872X6	B.F. Goodrich Chemical Co.
Nacrylic X4260	National Starch and Chemical Corp.
Nacrylic X4401	National Starch and Chemical Corp.
Nacrylic X4445	National Starch and Chemical Corp.
Nu-Film 17	Miller Chemical
Polyhall 295	Stein, Hall and Co., Inc.
Plyac	Allied Chemical Co.
Texcryl 3662	Poly-Acryls, RA Chemical Corp.
Ultra	Sanico
X-Link 2873	National Starch and Chemical Corp.